IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 7260
Fernando DOÑATE *et al.*	Art Unit: 1653
	Examiner: Anand Desai
Serial. No. 10/661,784	
Filed: September 15, 2003	Atty. Docket No. 28932.0006
For: HUMAN KININOGEN D3 DOMAIN POLYPEPTIDE AS AN ANTI-ANGIOGENIC AND ANTI-TUMOR AGENT	Customer No. 30827

DECLARATION OF ANDREW P. MAZAR PURSUANT TO 37 C.F.R § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, the undersigned, declare as follows:

1. I am a co-inventor of the above-referenced patent application, and am familiar with its contents.

2. I am Chief Scientific Officer of Attenuon LLC, in San Diego, CA. I have a Ph.D. in Biochemistry from the University of Illinois College of Medicine and am considered an expert in the field of protein chemistry. I have been involved for 16 years in drug discovery and development efforts. In over seven years at Abbott Laboratories, I initiated two anti-angiogenesis pilot projects and was involved in the pre-clinical development of two protein biological agents that are currently in Phase III trials. Subsequently, I invented or co-invented of a number of anti-angiogenic peptides one of which is currently in Phase II clinical trials. I am a co-inventor of a total of seven issued patents and over twenty pending patent applications and a member of the editorial board of *Recent Patent Reviews on Anticancer Drug Discovery.* I am the author or co-author of 57 full publications in refereed scientific journals (and 4 book chapters) including reviews on the uPA system, mouse tumor models and the therapeutic targeting of integrins. I am a frequent member of various NIH, VA, American Heart Association and other study sections.

3. I have read the pending Office Action in the above application, and understand the prior art rejections made over two references: Keith McCrae, WO 00/35407 ("McCrae") and Zhang *et al., Can. J. Physiol. Pharmacol. 80*:85-90 (2002) ("Zhang"). I am intimately familiar with these references, and the peptides disclosed therein, at least in part because:

(a) I am a close colleague and collaborator of Keith McCrae; indeed he is a co-inventor with me on several patent applications owned by my company; and

(b) I am a co-author of the Zhang reference (as is Keith McCrae).

4. As I understand it, the anticipation (and obviousness) rejections of the present claims are based on the alleged disclosure by McCrae of the inhibition of angiogenesis by high MW kininogen domain 3 (HK-D3) peptides including those that contain amino acid sequences Asn^{275}-Lys^{282}, Cys^{246}-Cys^{249}, and Leu^{331}-Tyr^{338}. These peptides allegedly "inhibit endothelial cell proliferation and are useful as anti-angiogenic agents" (Office Action citing page 14, lines 20-28).

5. As I understand it, the highly related rejection over Zhang touches upon two points:

(a) Zhang discloses the inhibition of endothelial cell (EC) cell proliferation by two-chain high MW kininogen (Results section, 1st paragraph, and Table 1). These larger polypeptides are distinct from those claimed in the present application - *i.e.*, molecules substantially no larger than the D3 domain.

(b) Secondly, Zhang discloses the inhibition of EC proliferation by two HK-D3 peptides and the induction of EC apoptosis by one of them (*Results* section, last paragraph; Figure 5). These two peptides correspond to amino acids 267-282 and 275-290 of HK. (Results section, last paragraph, and Figure 5.

> H3-6 is a 16mer with the sequence ^{267}TITKLNAENNATFYFK^{282} (same as SEQ ID NO:9 in McCrae)
>
> H3-7 is a 16mer with the sequence ^{275}NNATFYFKIDNVKKAR^{290} (half of which overlaps with H3-6; underscored in both. It is identical to SEQ ID NO: 10 in McCrae)

6. We know based on my own experimental data (that will be provided to the Examiner if requested) that the biological activity of these two peptide fragments of D3 as studied and disclosed in both McCrae and Zhang are artifacts of precipitation. These peptides were said by McCrae to have IC_{50} values against EC's of <0.8 μM (Table 1, p. 24). We now

know that these peptides are extremely insoluble in aqueous buffer, and the reason for this measured IC_{50} is that these peptides non-specifically stick to serum proteins in culture medium causing them to precipitate on cells, thereby inducing apoptosis non-specifically. So, of course, this non-specific, non-pharmacologic "side effect" would be observed in a EC proliferation assay as "inhibitory" and in an apoptosis assay as "apoptosis-inducing". We can demonstrate serum protein precipitation with a dose-response mirroring the "anti-proliferative" dose-response. It is not possible to test these peptides under serum free conditions since EC's spontaneously apoptose in the absence of serum. However, these peptides were tested under serum free conditions in very short-term assays that measure their effects on pathways known to be important for EC survival, e.g., the MAP kinase pathway and apoptotic pathways. Indeed, in such assays, these D3 peptides, in soluble form, had no effect on MAPK activation (the central pathway for EC proliferation) or the induction of apoptosis (measured as DNA fragmentation).

7. It is instructive to note that intact D3 inhibits EC proliferation and EC tube formation with an IC_{50} of ~200 nM. The only peptides actually tested in McCrae (Table 1, p. 24) had activities in the range of 28-44 μM, >100-fold less potent than the claims polypeptides in the EC proliferation assay. Thus, none of these peptides has the biological activity required by claim 1 ("at least 20% of the activity of native HK-D3"). Rather they have less than 1% of the activity.

8. In addition to the 16mer peptides from D3 discussed above, the specification refers explicitly to the McCrae reference at page 53, paragraph [0100], stating that:

> [0100] McCrae (WO 00/35407, June, 22, 2000)) described variants of the 8-mer peptide X_1-Asn-Asn-Ala-Thr-Phe-Tyr-Phe-Lys-X_2, (wherein X_1 and X_2 represented from zero to twelve additional amino acids of various sequence) that inhibit EC proliferation (and a cyclic Cys-Val-Gly-Cys peptide with a disulfide bond linking the two Cys residues, , which does not inhibit EC proliferation). These peptides are all sequences derived from the sequence of native HK-D3. The 8-mer peptide was asserted to inhibit EC with an IC_{50}<0.8 μM. However, the present inventors discovered that this peptide precipitated serum proteins present in serum supplement used in the cell proliferation assay, which led to apoptosis. Thus, what was reported by McCrae to be inhibition of proliferation was in fact an artifact of the precipitation phenomenon and subsequent induction of apoptosis. The actual IC_{50} of the above 8-mer peptide for true inhibition of EC proliferation was >50 μM. This is in contrast to HK-D3 (and its HK-D3v variant) which were more than 200-fold more inhibitory (IC_{50}~0.25 μM; see Figure 3). Thus, it is concluded that the shorter peptides described by McCrae are not sufficient to recapitulate the anti-proliferative activity of full length HK-D3 indicating either that (a) the peptides need to be conformationally constrained within the larger HK-D3 structure or (b) additional, previously unidentified regions of HK-D3 are required for the full inhibitory activity against ECs observed by the present inventors.

Just as with the 16mer peptides, these 8mer peptides behave artefactually because of their originally unrecognized insolubility under assay conditions.

9 The claims are directed to polypeptides that (a) are anti-angiogenic; (b) do not differ substantially in length from the full length HK-D3 domain, and, as presently claimed, can be modest variants from the native (or disclosed non-native) D3 sequences; and (c) are required to have at least 20% of the biological activity (not artefactual, non-biological activity) of native HK-D3). It is my opinion that none of the polypeptides disclosed in McCrae or in Zhang fit the above three conditions. Moreover, in addition to their weak binding activity, there is no reason to believe based on a knowledge of pharmacology and formulation that the 16mer or 8mer peptides disclosed in McCrae or Zhang would make suitable therapeutics or have any useful effects *in vivo* since, as discussed above, when they are in solution they have no biological action on EC's so that there is **no basis** for considering them anti-angiogenic. Hence, it is my opinion that those skilled in this field would **not agree** that the D3 peptides of McRae and Zhang discussed above are "anti-angiogenic polypeptides" despite the kind words lavished on them by the McCrae patent publication.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

4/28/05 _____

Date

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Andrew P. Mazar